



Interrelation of colouration and morphological traits in Northern Crested Newts (*Triturus cristatus*): towards a non-invasive tool for age determination

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Abstract. Determining animals' age is important for a wide range of study fields in biology. However, age determination is often problematic because it relies on invasive techniques. For the Northern Crested Newt (*Triturus cristatus*) various morphological traits are believed to be age-related. Quantifying interrelations among four morphological characteristics (snout–vent length, crest morphology, ventral colouration, tail base spot size), our study demonstrated that the traits are interrelated within and across populations. Both genetic and plastic adaptation to local environmental conditions unlikely explain the observed trait correlations. Furthermore, we found significant differences in snout–vent length and ventral colouration between adult and juvenile/subadult Crested Newts. Thus, there is strong evidence that the studied traits are age-related. Even though the ontogenetically mediated trait variation is masked by a non-age-related variability, the analysis of morphological traits serves as a powerful tool for obtaining insights into Crested Newts' population demography in a non-invasive fashion.

Key words. Amphibia, Caudata, population demography, monitoring, snout–vent length, crest morphology, belly pattern, tail base spot size.

Introduction

Knowledge about animal population demography is crucial for species conservation (LANDE 1988, CLUTTON-BROCK & SHELDON 2010). However, age determination could be difficult if it relies on non-invasive techniques and is thus often restricted on dead individuals or premised on invasive methods (LAWS 1950, KLOMP & FURNESS 1992, HAYES & WALL 1999, SINSCH 2015). Skeletochronology is commonly used for determining age in amphibians (e.g. MIAUD et al. 1993, SINSCH 2015). However, when individuals exceed a certain age, precision of the estimates decreases dramatically because lines of arrested growth (LAGs) may resorb at a high rate (e.g. EDEN et al. 2007, SINSCH 2015). Furthermore, testis lobes have been used for estimating the age of male Crested Newts with some accuracy (DOLMEN 1983). As non-invasive methods for determining newt age are lacking so far, the age structure of populations is widely ignored in monitoring programmes although demography is a major determinant of population viability (GRIFFITHS

et al. 2010). In Crested Newt surveys evidence concerning population viability is usually based on presence/absence records or counts of eggs and larvae (e.g. English Nature 2001, BfN & BLAK 2015) but reproductive success can vary substantially among years (KUPFER & KNEITZ 2000) and the number of larvae in a single breeding season is therefore a weak indicator for population demography.

For Northern Crested Newts, *Triturus cristatus* (LAURENTI, 1768), a large variability has been reported in several morphological traits (DOLMEN 1983, ARNTZEN & TEUNIS 1993, MALMGREN & THOLLESSON 1999, BAKER & HALLIDAY 2000, KUPFER & VON BÜLOW 2011, PALAU DAVAL et al. 2018) and has been related to ontogeny in some of them (ARNTZEN & TEUNIS 1993, BAKER & HALLIDAY 2000, KUPFER & VON BÜLOW 2011, PALAU DAVAL et al. 2018). Principally, body size continually increases throughout the life of urodeles (KARA 1994) and snout–vent length has been shown to be age-related among Crested Newts (e.g. DOLMEN 1983, ARNTZEN 2000, SINSCH et al. 2003). However, annual growth rates are low in adult newts (ARNTZEN

2000, SINSCH 2015) and can differ significantly among years and environments (HAGSTRÖM 1980, CAETANO & CASTANET 1993, ARNTZEN 2000). Even for newts of the same age body sizes can differ considerably between different populations (see CAETANO & CASTANET 1993 for *T. marmoratus* or MEYER & GROSSE 2006 for *T. cristatus*) and individual variability in annual growth exceeds the age-related body size variation (SINSCH et al. 2003). Thus, directly extrapolating age from the body length of individuals is almost impossible.

BAKER & HALLIDAY (2000) drew attention to the variation in dorsal crest morphology for male Crested Newts of different age classes and recently PALAU DAVAL et al. (2018) confirmed previous findings: a digitate crest was predominant in the age class of first year breeders whereas an irregular crest morphology was more often observed in older newts. Simultaneously, the number of crest peaks tended to decrease with the age of male newts but the crest area increased relative to the body area with increasing age (PALAU DAVAL et al. 2018). Similarly, variability in the newts' belly pattern, characterised by black spots of different size and shape (Fig. 1), was found (e.g. ARNTZEN & TEUNIS 1993, KUPFER 1996, VON BÜLOW 2001). ARNTZEN & TEUNIS (1993) as well as GROSSE & GÜNTHER (1996) reported an increase in the number and size of black spots throughout the life of Crested Newt specimens from different geographical regions. For male Northern Crested Newts, a yellow spot at the tail base, posterior to the cloaca, is another colouration feature showing strong variability (Fig. 1). The size of the "tail base spot" apparently decreases throughout the newts' life (KUPFER & VON BÜLOW 2011).

Genetic drift and both genetic and plastic adaptation to local environmental conditions may result in trait variability between populations and genetic diversity can promote phenotypic diversity also within populations (CAETANO & CASTANET 1993, VAN BUSKIRK 2009, URBAN et al. 2014). Nevertheless, variation in the before mentioned traits has often been traced to ontogeny and we therefore hypothesise that morphological traits are interrelated independent of allometry. To test this hypothesis, we assess the correlations of snout-vent length, crest morphology, ventral colouration and the size of the tail base spot. Furthermore, we test whether a detailed assessment of these easily accessible morphological traits could allow insights into the population demography of endangered Crested Newts.

Material and methods

In total, 582 adult male, 575 adult female as well as 28 juvenile (one hibernation, sex undetermined) and 78 subadult (at least two hibernations, sex undetermined) individually different Northern Crested Newts (*Triturus cristatus*) were caught in the course of a population assessment at fourteen ponds in Baden-Württemberg (south-west Germany) during three breeding seasons from 2016 to 2018. Stages and sexes were distinguished based on the morphology of the newts' cloaca (e.g. PALAU DAVAL et al. 2018). For male newts,

the morphology of the dorsal crest (CM) was recorded visually distinguishing three different categories of crest morphology, following the classification of BAKER & HALLIDAY (2000): 1 (digitate), 2 (intermediate, including dentate) and 3 (irregular). The belly pattern was recorded for all stages and sexes using digital compact cameras (Nikon Coolpix S7000, Panasonic Lumix DMC-FX01) and a self-constructed apparatus for standardized imaging ($27 \times 17 \times 45$ cm) including a glass pane and a scale bar. A piece of foam placed in top of the newts prevented specimens from vertical and horizontal movements during imaging. We measured snout-vent length (SVL) based on the photographs of the newts' venters to the nearest 1.0 mm from the tip of the snout to the posterior edge of the cloaca. For measuring snout-vent length and to extract the belly pattern the software AmphIdent was used (MATTHÉ et al. 2008). Subsequently, the percentage of dark ventral colouration (DVC)



Figure 1. Example of a male Northern Crested Newt (*Triturus cristatus*) bearing a large tail base spot (indicated by the black arrow) and 59% of dark ventral colouration.

was calculated by means of Adobe® Photoshop® CS2 and ImageJ (RASBAND, W.S. 1997–2018). According to the histogram of the photographs a certain pixel value was set as a threshold to distinguish black and yellow parts of the belly pattern. The size of the male newts' tail base spot (TBSS) was classified visually based on the photographs of the newts' venter. Four different categories were distinguished: A (no spot), B (small), C (medium), D (large).

Statistical analyses were performed using RStudio with R 3.5.1 (RStudio Team 2016, R Development Core Team 2018). We used a three-step approach for investigating the interrelations of different morphological traits. Using correlation analyses, we studied interrelations between SVL and DVC. We performed a χ^2 -test to check correlations between CM and TBSS and one-way analyses of variance (ANOVA) for investigating the interrelations between a metric response and a categorical explanatory variable. Factor levels with significant differences were identified by Tukey-style pairwise comparisons. Benjamini-Hochberg correction was used to adjust p-values for multiple comparisons (BENJAMINI & HOCHBERG 1995). Second, linear models explaining snout-vent length and the percentage of dark ventral colouration by population means were performed and the before mentioned analyses were repeated using the residuals of the linear models as response variable. Thus, we ensured that the observed interrelations are not simply the result of differences between populations but also exist within populations. Third, we used linear mixed models to assess the relative strength of trait correlations and to check for additional trait-environment correlations between the four morphological characters under study and two environmental variables: elevation and percent plant cover (PC) of the newts' breeding pond. Setting "population" as random factor, we controlled for effects of population membership. Model selection was based on AICc-values, Akaike weights (w_i) and evidence ratios (AKAIKE 1973, WAGENMAKERS & FARRELL 2004). For the best supported models consisting only of morphological explanatory variables, we calculated marginal and conditional R^2 -values (NAKAGAWA & SCHIELZETH 2013). R^2_{marginal} describes the proportion of variance which is explained by the fixed effects (morphological traits), $R^2_{\text{conditional}}$ the proportion that is explained by fixed plus random effects (RUPPERT et al. 2015), i.e. by morphological traits plus the variation among populations. Finally, we tested for stage-dependency of snout-vent length and ventral colouration using linear mixed models with the newts' stage (juvenile, subadult, adult) as fixed and "population" as random factor.

Results

We found clear correlations between the studied morphological traits. Snout-vent length differed considerably between male newts of all crest shape categories and was highest for individuals showing irregular crest morphologies (ANOVA, $F_{2,579} = 68.18$, p-value < 0.001, Table 1, Fig. 2A). Furthermore, snout-vent length varied in accord-

Table 1. Snout-vent length (SVL) and proportion of dark ventral colouration (DVC) in male Northern Crested Newts (*Triturus cristatus*) of different crest morphology (CM) and tail base spot size (TBSS). Across-population estimated differences in SVL (mm) and DVC (proportion), t-values and Benjamini-Hochberg-adjusted p-values are reported. Factor levels of crest morphology are: 1 (digitate), 2 (intermediate, including dentate), 3 (irregular). For the size of the tail base spot the factor levels are: A (no spot), B (small), C (medium), D (large).

response variable	explanatory variable	pairwise comparisons			
		levels	difference	t	p-value
SVL	CM	1–2	-3.77	- 6.36	< 0.001
		1–3	-8.48	-11.65	< 0.001
		2–3	-4.72	-7.86	< 0.001
SVL	TBSS	A–B	2.97	5.32	< 0.001
		A–C	6.03	8.88	< 0.001
		A–D	7.33	10.19	< 0.001
		B–C	3.07	4.27	< 0.001
		B–D	4.36	5.78	< 0.001
		C–D	1.30	1.52	0.129
DVC	CM	1–2	-0.04	-2.71	< 0.05
		1–3	-0.06	-3.25	< 0.01
		2–3	-0.02	-1.27	0.204
DVC	TBSS	A–B	0.09	7.35	< 0.001
		A–C	0.13	8.94	< 0.001
		A–D	0.15	10.08	< 0.001
		B–C	0.04	2.75	< 0.01
		B–D	0.07	4.16	< 0.001
		C–D	0.02	1.37	0.170

ance with the size of the tail base spot. The smaller the tail base spot, the larger snout-vent lengths were found (ANOVA, $F_{3,578} = 48.35$, p-value < 0.001, Table 1, Fig. 2B). The proportion of dark ventral colouration differed in accordance with crest morphology and tail base spot size. Males with irregularly shaped dorsal crests showed the highest percentage of dark ventral colouration (ANOVA, $F_{2,579} = 5.71$, p-value < 0.01, Table 1, Fig. 2C) and the percentage of dark ventral colouration decreased with increasing size of the tail base spot (ANOVA, $F_{3,578} = 50.23$, p-value < 0.001, Table 1, Fig. 2D). Moreover, we observed a significant interrelation of crest morphology and tail base spot size (χ^2 -test, $\chi^2_6 = 57.30$, p-value < 0.001). Tail base spots were missing in 56% of male newts having an irregularly shaped dorsal crest ($n = 116$) and in less than 7% of the newts within this crest shape category medium-sized or large spots were observed. By contrast, males with digitate dorsal crests ($n = 120$) had tail base spots of medium or large size in 46% and none in only 20% (Fig. 3). A high proportion of dark ventral colouration was related to large body sizes in both male and female Northern Crested Newts (Correlation analyses, $r = 0.31$, $F_{1,580} = 63.41$, p-value < 0.001 and $r = 0.37$, $F_{1,573} = 10.37$, p-value < 0.001 for males and females, respectively, Fig. 4).

Results were similar when we controlled for population-specific effects. We found significant within-population interrelations between snout–vent length and crest morphology (ANOVA, $F_{2,579} = 65.86$, p -value < 0.001) as well as between snout–vent length and the size of the tail base spot (ANOVA, $F_{3,578} = 29.14$, p -value < 0.001). We found the largest body sizes in male newts with irregularly shaped crests and no tail base spots (Table 2). Ventral colouration was significantly related to crest morphology (ANOVA, $F_{2,579} = 6.18$, p -value < 0.01 , Table 2) and tail base spot size (ANOVA, $F_{3,578} = 18.53$, p -value < 0.001 , Table 2) of male Crested Newts after controlling for variation between populations. The darkest belly patterns were observed in male newts with an irregular crest morphology and without a tail base spot. Furthermore, we found a significant within-population correlation of snout–vent length and the proportion of dark ventral colouration (Correlation analyses, $r = 0.11$, $F_{1,580} = 6.66$, p -value < 0.05 and $r = 0.21$,

$F_{1,573} = 26.63$, p -value < 0.001 for male and female Crested Newts, respectively, Fig. 4). However, correlation coefficients decreased compared to the previous correlation analyses indicating that the overall trait correlation results from both interrelations within and between populations.

Among the studied traits, linear mixed models indicated strongest interrelations between crest morphology and snout–vent length as well as between tail base spot size and ventral colouration (Table 3, Table 4). For ventral colouration, the model using tail base spot size as single fixed explanatory variable was best supported. However, the large difference between $R^2_{\text{conditional}} = 0.52$ and $R^2_{\text{marginal}} = 0.08$ indicated that the variability in male newts' ventral colouration was probably larger in response to the population membership compared to the different tail base spot sizes. Neither additional morphological nor environmental variables improved model likelihood (Table 3). In contrast, all three morphological variables (DVC, CM, TBSS) contrib-

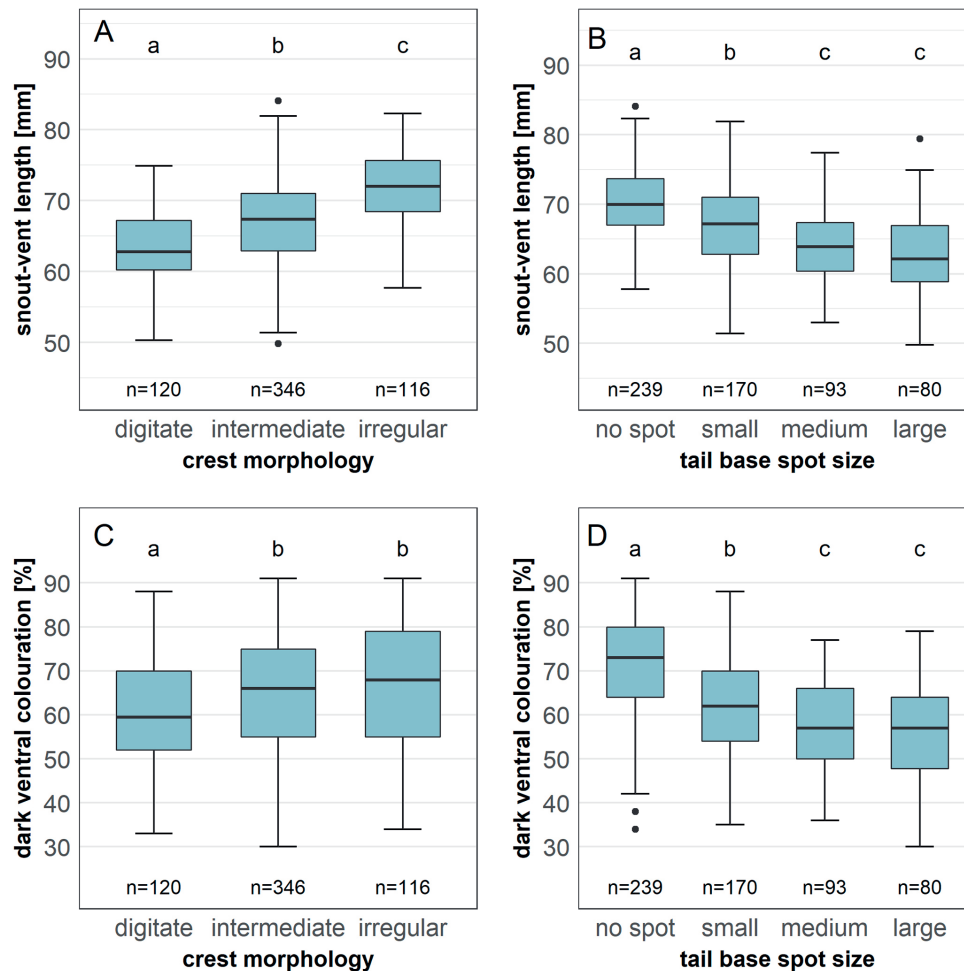


Figure 2. Interrelations of four morphological traits in male Northern Crested Newts (*Triturus cristatus*). A) Median male snout–vent length with variable crest morphologies. B) Median male snout–vent length with different tail base spot sizes. C) Proportion of dark ventral colouration of male newts having different crest morphologies. D) Proportion of dark ventral colouration of male newts having variable tail base spot sizes. The box indicates the median and interquartile range (IQR). Whiskers are drawn to the furthest data point within a $1.5 \times \text{IQR}$ from the box. Black dots indicate distant outliers of more than $1.5 \times \text{IQR}$ and different letters denote factor levels which differ significantly ($\alpha = 0.05$) according to pairwise comparisons.

uted substantial unique information to the model of male newts' snout-vent length (Table 4). $R^2_{\text{conditional}} = 0.44$ and $R^2_{\text{marginal}} = 0.31$ differ only slightly, indicating that variation in snout-vent lengths is in the first place explained by variability in the other morphological traits. Models did not improve when elevation was added as additional explanatory variable (statistical information not shown here) but models testing percent plant cover of the newts' breeding pond as additional explanatory variable were highly supported. Evidence ratios suggested that, for explaining male Northern Crested Newts' snout-vent lengths, a model including percent plant cover of the breeding pond (model 2.5) is slightly more likely than a model containing morphological variables only (model 2.1) and thus highlighted the potential influence of environmental factors on morphological traits. However, model likelihood decreased more strongly when morphological explanatory variables were removed. Dropping CM, TBSS or DVC from the full model reduced model likelihood by more than factor 10^{21} , 10^{13} and 1.4, respectively, indicating that interrelations between the studied traits tended to be stronger than trait-environment correlations. The best supported model (model 2.5) suggested highest snout-vent lengths for male newts that inhabit a densely vegetated pond and have no tail base spot, a dark belly pattern and an irregularly shaped dorsal crest.

Generally, our analyses revealed clear interrelations between the four studied traits both within and across populations. Whereas variability in snout-vent length was in the first place associated with variation in other morphological traits, variation in the ventral colouration of male Crested Newts was to a great extent associated with inter-popula-

Table 2. Snout-vent length (SVL) and proportion of dark ventral colouration (DVC) in male Northern Crested Newts (*Triturus cristatus*) of different crest morphology (CM) and tail base spot size (TBSS). Within-population estimated differences in SVL (mm) and DVC (proportion), t-values and Benjamini-Hochberg-adjusted p-values are reported. Factor levels of crest morphology are: 1 (digitate), 2 (intermediate, including dentate), 3 (irregular). For the size of the tail base spot the factor levels are: A (no spot), B (small), C (medium), D (large).

response variable	explanatory variables	pairwise comparisons			
		levels	difference	t	p-value
SVL	CM	1-2	-3.14	-5.85	< 0.001
		1-3	-7.53	-11.40	< 0.001
		2-3	-4.38	-8.06	< 0.001
		A-B	2.09	3.98	< 0.001
		A-C	4.31	6.72	< 0.001
SVL	TBSS	A-D	5.45	8.05	< 0.001
		B-C	2.21	3.27	< 0.01
		B-D	3.35	4.72	< 0.001
		C-D	1.14	1.43	0.154
DVC	CM	1-2	0.01	0.69	0.494
		1-3	-0.02	-2.33	< 0.05
		2-3	-0.03	-3.51	< 0.01
		A-B	0.02	2.67	< 0.05
		A-C	0.05	4.92	< 0.001
DVC	TBSS	A-D	0.07	6.70	< 0.001
		B-C	0.03	2.58	< 0.05
		B-D	0.05	4.40	< 0.001
		C-D	0.02	1.73	0.084

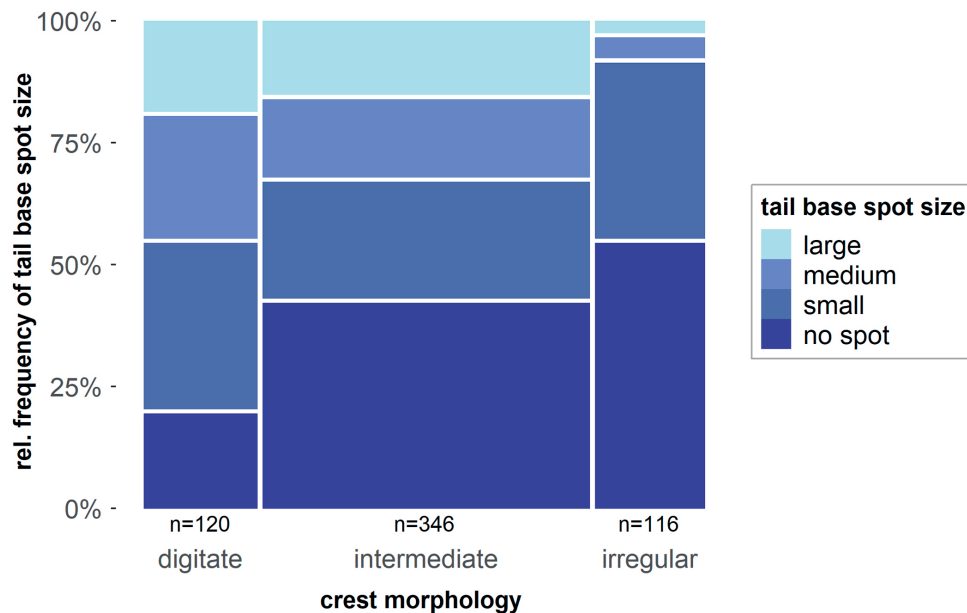


Figure 3. Relative frequency of tail base spot size categories depending on male Northern Crested Newts' (*Triturus cristatus*) crest morphology (n = 582 males).

Table 3. Linear mixed models explaining the proportion of dark ventral colouration in male Northern Crested Newts (*Triturus cristatus*). The first four models use morphological variables only to explain snout–vent length. Models 1.5 and 1.6 include plant cover (PC) of the newts' breeding pond.

model	response variable	explanatory variables (fixed)	explanatory variable (random)	AIC _c	Δ_{AICc}	w _i
1.1	DVC	TBSS	Population	-1278.01	0.00	5.78*10 ⁻¹
1.2	DVC	CM+SVL+TBSS	Population	-1251.26	26.76	8.96*10 ⁻⁷
1.3	DVC	SVL	Population	-1240.15	37.87	3.46*10 ⁻⁹
1.4	DVC	CM	Population	-1231.33	46.68	4.22*10 ⁻¹¹
1.5	DVC	TBSS+PC	Population	-1277.38	0.63	4.22*10 ⁻¹
1.6	DVC	PC	Population	-1238.36	39.65	1.42*10 ⁻⁹

Table 4. Linear mixed models explaining the snout–vent length of male Northern Crested Newts (*Triturus cristatus*). The first four models use morphological variables only to explain snout–vent length. Models 2.5 to 2.9 include plant cover (PC) of the newts' breeding pond.

model	response variable	explanatory variables (fixed)	explanatory variable (random)	AIC _c	Δ_{AICc}	w _i
2.1	SVL	TBSS+CM+DVC	Population	3488.74	1.60	2.55*10 ⁻¹
2.2	SVL	CM	Population	3564.25	77.09	1.03*10 ⁻¹⁷
2.3	SVL	TBSS	Population	3594.31	107.16	3.04*10 ⁻²⁴
2.4	SVL	DVC	Population	3675.64	188.49	6.65*10 ⁻⁴²
2.5	SVL	TBSS+CM+DVC+PC	Population	3487.15	0.00	5.66*10 ⁻¹
2.6	SVL	TBSS+CM+PC	Population	3489.45	2.30	1.79*10 ⁻¹
2.7	SVL	CM+DVC+PC	Population	3548.65	61.50	2.50*10 ⁻¹⁴
2.8	SVL	TBSS+DVC+PC	Population	3589.52	102.36	3.35*10 ⁻²³
2.9	SVL	PC	Population	3693.87	206.72	7.32*10 ⁻⁴⁶

tion variability. We related ventral colouration and snout–vent length to the newts' stage and found that snout–vent lengths (Linear mixed model, $\chi^2_2 = 756.97$, p-value < 0.001)

and proportions of dark ventral colouration (Linear mixed model, $\chi^2_2 = 113.66$, p-value < 0.001, Fig. 5) differed between stages. Body size increased significantly from juve-

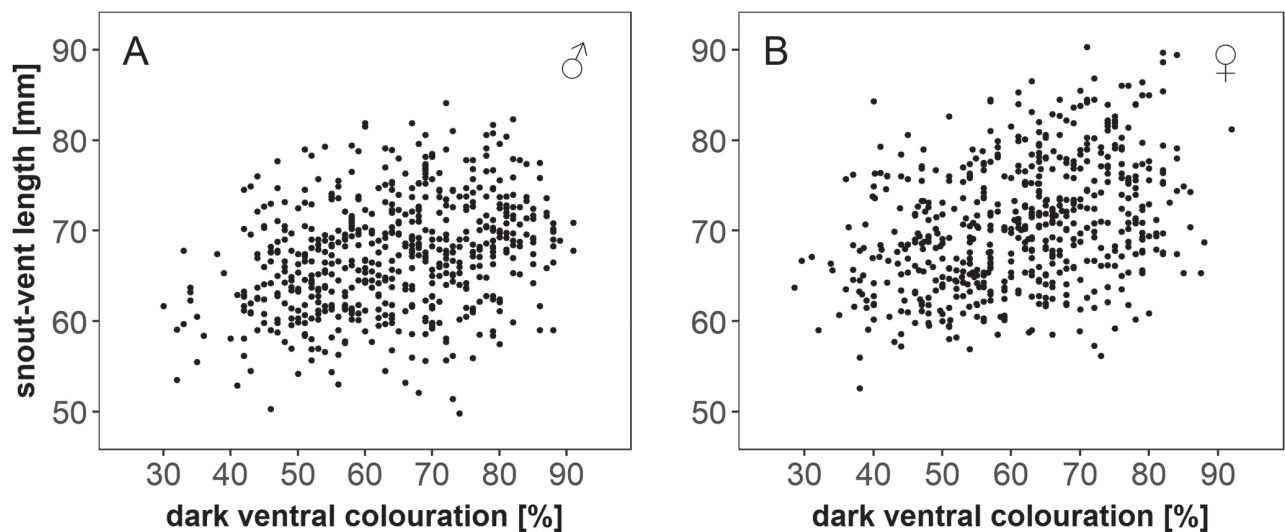


Figure 4. Correlation of snout–vent length and proportion of dark ventral colouration for Northern Crested Newts (*Triturus cristatus*) A) in male ($r = 0.31$, p-value < 0.001) and B) in female newts ($r = 0.37$, p-value < 0.001). In both sexes snout–vent length and the percentage of dark ventral colouration were positively correlated.

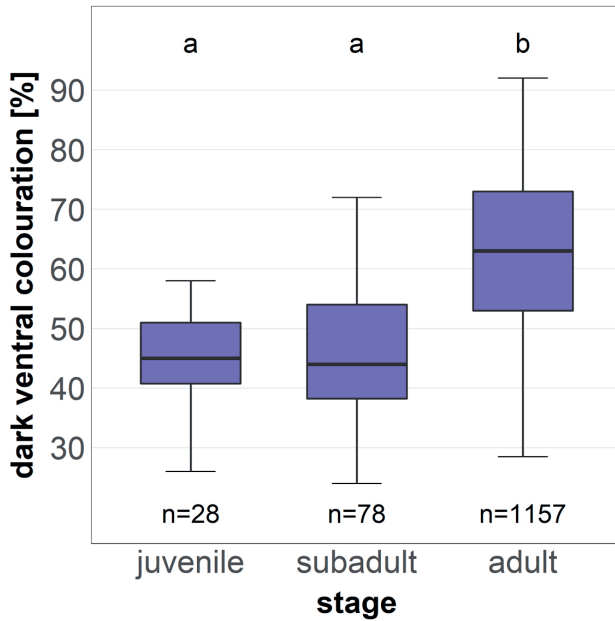


Figure 5. Percentage of dark ventral colouration in Northern Crested Newts (*Triturus cristatus*) of different stages. The box indicates the median and interquartile range (IQR). Whiskers are drawn to the furthest data point within a $1.5 \times \text{IQR}$ from the box. Different letters denote factor levels which differ significantly ($\alpha = 0.05$) according to pairwise comparisons.

nile to adult newts (Pairwise comparisons, juvenile – subadult: -8.86 , $z = -6.72$, $p\text{-value} < 0.001$; juvenile – adult: -23.40 , $z = -20.25$, $p\text{-value} < 0.001$; subadult – adult: -14.54 , $z = -20.04$, $p\text{-value} < 0.001$) and belly patterns of adult Crested Newts were significantly darker than that of juveniles and subadults (Pairwise comparisons, juvenile – subadult: -0.01 , $z = 0.30$, $p\text{-value} = 0.767$; juvenile – adult: -0.10 , $z = -6.12$, $p\text{-value} < 0.001$; subadult – adult: -0.10 , $z = -9.16$, $p\text{-value} < 0.001$).

Discussion

We found significant interrelations among different morphological traits both in male and female Northern Crested Newts. Our results demonstrated that trait interrelations are not simply the result of variability between populations but do also exist within populations. Thus, genetic drift as well as genetic and plastic adaptation to local environmental conditions cannot fully account for the observed correlations. Ontogenetically mediated trait variation, as previously proposed by several researchers (e.g. ARNTZEN & TEUNIS 1993, GROSSE & GÜNTHER 1996, BAKER & HALLIDAY 2000, KUPFER & VON BÜLOW 2011, PALAU DAVAL et al. 2018), could explain the observed trait correlations within and across populations. We found a significant correlation of both snout–vent length and the darkness of the newts’ belly pattern with the stage of Northern Crested Newts. Therefore, our results do not only confirm

the hypothesis that the studied morphological traits are interrelated but do also indicate that they might principally be useful for estimating the age of Crested Newts. However, we acknowledge that our results do not indisputably confirm that the studied traits vary in accordance with age within adult Crested Newts. Growth of newts slows down after they reached maturity (ARNTZEN 2000) and the darkness of the belly pattern might not increase linearly with age.

Although Crested Newt body sizes vary geographically (ARNTZEN 2003) there is no consistent relationship between body size and climatic gradients (e.g. ARNTZEN 2000, KRIZMANIC et al. 2005, FICETOLA et al. 2010, this study). Besides age and climatic conditions, genetics, food availability, newt and predator density as well as foraging behaviour may influence newt growth (ARNTZEN 2000). Our models indicated that dense vegetation in the newts’ breeding pond may positively affect body size of Crested Newts. Dense vegetation likely favours aquatic invertebrates and therefore increases food availability for larval and mature Crested Newts (OLDHAM et al. 2000). Generally, snout–vent length is considered a weak indicator of amphibians’ age. SINSCH et al. (2003) drew attention to a considerable individual variation in annual growth which is much stronger than the variability between different age classes. Individuals might mature later while growing slower and thus finally reach a larger body size than fast growing individuals – a scenario highlighted by KUHN (1994) for the common toad (*Bufo bufo*). Nevertheless, even though individual snout–vent length is strongly influenced by various factors, there is undoubtedly a general trend of increasing snout–vent length with increasing age (e.g. ARNTZEN 2000, SINSCH et al. 2003). Ontogenetic change of colouration patterns has been observed in several salamandrids such as *Salamandra corsica* (BEUKEMA 2011), *Salamandra s. gallaica*, *S. s. crespoides* and *S. algira tingitana* (BOGAERTS 2002 cited in BEUKEMA 2011) as well as *Triturus marmoratus* (CAETANO & CASTANET 1993). However, a strong environmentally mediated variation in ventral or dorsal colouration pattern development was reported (CAETANO & CASTANET 1993, BEUKEMA 2011). We found considerable variability in the ventral colouration within juvenile and subadult Crested Newts even though they did not differ in age (Fig. 5). Furthermore, we found a strong effect of population membership on the ventral colouration of adult male Northern Crested Newts. These findings indicate that the role of genetics and plasticity should not be neglected aiming to explain variation in morphological traits. Nonetheless, our analyses proved that the percentage of dark ventral colouration correlates with other morphological traits and therefore suggested age-relatedness.

PALAU DAVAL et al. (2018) confirmed the hypothesis of BAKER & HALLIDAY (2000) that the morphology of Northern Crested Newts’ dorsal crests varies between male newts of different age classes. Furthermore, they report a decrease in the number of crest peaks with age, an increase of the crest area relative to the body area and an increase in size of the reflective white spot on the side of the male newts’

tails. Our results demonstrate that crest morphology varies in accordance with snout–vent length, ventral colouration and tail base spot size.

We demonstrated that variability in each studied morphological trait can to a certain extent be explained by variation in other morphological traits. As traits are interrelated within and across populations and as it appears very unlikely that all four morphological traits are genetically linked on the level of individuals, we found high evidence for an ontogenetically mediated variability in snout–vent length, ventral colouration, crest morphology and tail base spot size in Northern Crested Newts. Thus, the studied traits are principally useful for assessing the age of Northern Crested Newts and likely their close relatives. At least for the Italian Crested Newt (*Triturus carnifex*) the investigated traits seemed to be interrelated in a similar fashion (H. HINNEBERG unpubl. data). However, as indicated for snout–vent length and ventral colouration, correlations among morphological traits, and likely correlations between traits and age, are to a varying extent masked by non-age-related variability (for SVL see also SINSCH et al. 2003). Furthermore, the age indication of different traits was not always consistent on the level of a single individual. As an example, a male Crested Newt showing an irregular crest morphology and large body size (indicating old age) but a large tail base spot and only small proportion of dark ventral colouration (indicating young age) was included in our study. Using morphological traits for estimating the age of individual newts does therefore not appear to be sensible on the basis of our current knowledge. However, we suggest that estimating population age structure based on morphological traits is feasible if the number of newts caught is sufficient, and if information on multiple age-related traits is combined. In our study, we sampled 113 individually different male Crested Newts from the same population. The median snout–vent length for this population (63 mm) was significantly smaller than the median snout–vent length across all studied populations (67 mm) and the median percentage of dark ventral colouration was 50% compared to 64% across all populations. In this particular population, male newts with a digitate crest morphology were much more abundant (32%) than across all populations (21%), and males having large or medium-sized tail base spots occurred frequently (54% compared to 30% across all sampled populations). Thus, we conclude that young individuals had a high proportion in this population.

Assessing different ontogenetically mediated traits together has the potential to provide insight into the population demography of Crested Newts, even though each trait might show considerable non-age-related variation. Future research focusing on the interrelations of various morphological traits with age could contribute to support external morphological traits as a reliable and very powerful non-invasive tool. Trait analysis could be easily applied in monitoring programmes (e.g. VON BÜLOW & KUPFER 2019) when information about the demography of Crested Newt populations is desired.

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